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Reviewer A

Emerging evidence suggests that PARP inhibitor therapy increases the risk for therapy-related myeloid neoplasms. In the manuscript entitled "Therapy-related myeloid neoplasms in Korean patients with ovarian or primary peritoneal cancer treated with poly(ADP-ribose) polymerase inhibitors", the authors present a single-institution retrospective study on the incidence of therapy related myeloid neoplasms in 53 Korean patients with ovarian cancer or primary peritoneal cancer treated with PARP inhibitors. 5/53 patients develop t-MN, all of which have complex karyotype and/or TP53 mutations.
While the conclusions are limited by the small patient number and the retrospective design, this study addresses an important research question. I have the following suggestions for the authors to improve the manuscript:

- P.2 l. 21 instead of TP53 expression it should say "TP53 mutations"

Response: Thank you for your careful review. We have revised the phrase accordingly.

"Next-generation sequencing performed in four patients revealed *TP*53 mutations and complex karyotypes in all tested patients." (Abstract, Page 3, Line 15-16)

- P.3 l. 60. DNA damage caused by cytotoxic treatment is not the only mechanism for the emergence of t-MNs. It has become clear in recent years, that in many cases, the positive selection of pre-existing TP53-mutated clonal hematopoiesis is the origin of t-MNs. (PMID: 25487151, PMID: 25487151, PMID: 25487151, PMID: 25487151). This should also be included in the discussion.

Response: We agree with your suggestion. Accordingly, we have added the following sentences regarding the potential association of preexisting CHIP variants, including *TP53* mutations, with the development of t-MNs after PARPi treatment in the Discussion section.

"Furthermore, several studies have reported an association between preexisting clonal hematopoiesis of indeterminate potential (CHIP) variants and the development of t-MNs following PARPi treatment (26, 27,

28). These studies suggest that PARPi therapy imposes selective pressure, leading to the enhanced expansion of specific clones, particularly those with *TP53* mutations (27). Therefore, performing NGS to identify CHIP variants before starting PARPi treatment could provide a clearer understanding of the associated risks."

(Discussion, Page 13, Line 14-19)

Ref)

26. Kwan TT, Oza AM, Tinker AV, et al. Preexisting TP53-variant clonal hematopoiesis and risk of secondary myeloid neoplasms in patients with high-grade ovarian cancer treated with Rucaparib. JAMA Oncol 2021;7:1772-81.

27. Martin JE, Khalife-Hachem S, Grinda T, et al. Therapy-related myeloid neoplasms following treatment with PARP inhibitors: new molecular insights. Ann Oncol 2021;32:1046-8.

28. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature 2015;518:552-5.

- P.4 l. 79. Listing t-MN diagnosis as an inclusion criterion is misleading, as it suggests only patients that developed t-MN were included in this retrospective study.

Response: Thank you for pointing out this discrepancy. Accordingly, we have revised the sentence related to t-MN diagnosis as follows.

"patients diagnosed with t-MNs after PARPi therapy must meet diagnostic criteria for t-MNs according to the 2016 or 2022 World Health Organization classification"

(Methods, Page 7, Line 16-18)

- Methods: p.5 l. 100: please include details about the NGS workflow that was used.

Response: Thank you for the comment. Accordingly, the following sentence has been added to the Methods section:

"next-generation sequencing (NGS) was conducted on BM samples to investigate genomic alterations in 49 cancer-associated myeloid neoplasm genes. This process included nucleic-acid isolation, library preparation, sequencing, and data analysis."

(Methods, Page 8, Line 12-14)

- In the methods section, the authors state that Cox regression models were used to identify prognostic factors for t-MN occurrence. In the results section, however, the results of a logistic regression are reported. The way I understand the authors intention, is to identify prognostic factors for t-MN occurrence in patients that are being treated with PARPi. I suggest running a multivariable Cox proportional hazards model with t-MN occurrence as event and censored on deaths/lost-to follow up from the time when PARPi therapy was started. Aside from age, BRCA status and history of other cancer types, the most important covariate would be the 'cumulative dose' of cytotoxic (in particular platinum) and radiation therapy administered before initiation of PARPi treatment.

Response:

1> In our study, we initially conducted logistic regression analysis but incorrectly described the method as Cox regression models in the Methods section. However, following the reviewer's recommendation, we have determined that using Cox regression models is a more appropriate analytical approach. Therefore, we re-conducted the analysis using Cox regression models and have updated the results accordingly.

2> As noted by the reviewer, we intended to analyze the cumulative dose of cytotoxic chemotherapy and radiation therapy before PARPi treatment. However, owing to the lack of data in our institution's EMR, we were unable to perform this analysis. We have added this limitation to the Discussion section.

"In the univariable analyses, age at ovarian or primary peritoneal cancer (hazard ratio [HR], 0.856; 95% confidence interval [CI], 0.681–1.075; p=0.180), PARPi usage period (HR, 0.958; 95% CI, 0.900–1.021; p=0.184), and OS from ovarian cancer diagnosis (HR, 1.251; 95% CI, 0.958–1.635; p=0.101) were significantly associated with t-MN occurrence. However, in the multivariable analysis, none of these associations remained statistically significant (Table 3)."

(Results, Page 10, Line 8-12)

"Additionally, the occurrence of t-MNs is notably influenced by prior treatment history. Therefore, including the cumulative doses of cytotoxic chemotherapy and radiotherapy in the analysis would have been valuable. However, owing to missing data for some patients, this analysis could not be performed." (Discussion, Page 14, Line 3-6)

- Please include the type of PARPi administered in table 1 as t-MN incidence rates seem to differ depending on the agent used. Also, the reason why PARPi therapy was discontinued (progressive disease, side effects…) should be reported.

Response: Thank you for the comment. In our study, the types of PARPi administered to the included patients were olaparib and niraparib. Olaparib was administered to 25 patients (48.0%), and niraparib was administered to 27 patients (51.9%). Additionally, all 4 patients diagnosed with t-MNs were treated with olaparib. Among patients who discontinued PARP inhibitor treatment, the most common reason was disease progression (PD) in 28 patients (53.8%), while discontinuation due to side effects occurred in 2 patients (4.2%). All 4 patients with t-MNs discontinued treatment owing to PD. This information has been added to the following sentence and Table 1.

"The types of PARP inhibitors administered were olaparib for 25 patients (48.0%) and niraparib for 27 patients (51.9%). All 4 patients diagnosed with t-MNs were treated with olaparib." (Results, Page 9, Line 12-14)

- In Figure 1, it would be informative if the other previous cytotoxic treatment lines also be displayed

Response: Thank you for this recommendation. We have added descriptions of other previous cytotoxic treatment lines to Figure 1.

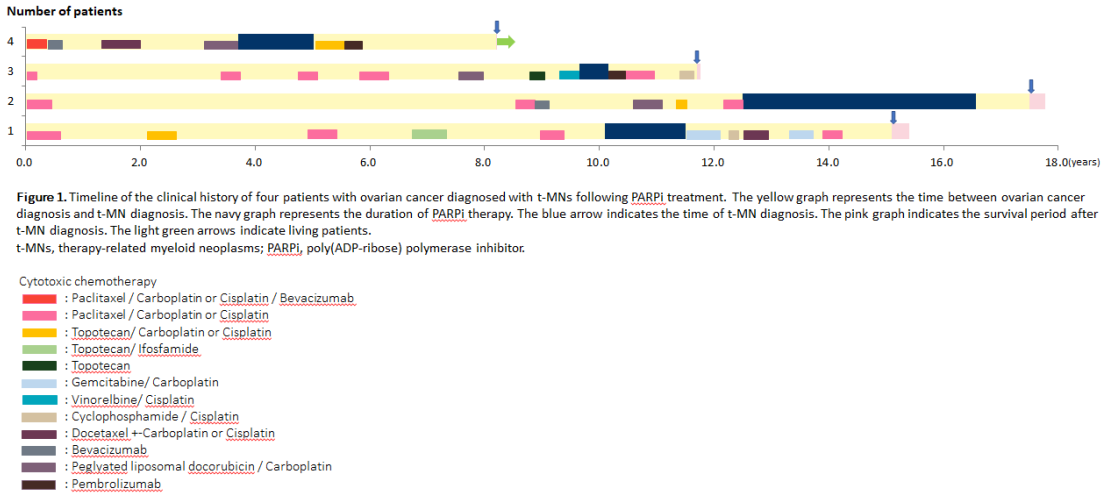

Figure 1. Timeline of the clinical history of four patients with ovarian cancer diagnosed with t-MNs following PARPi treatment. The yellow graph represents the time between ovarian cancer diagnosis and t-MN diagnosis. The navy graph represents the duration of PARPi therapy. The blue arrow indicates the time of t-MN diagnosis. The pink graph indicates the survival period after t-MN diagnosis. The light green arrows indicate living patients.
t-MNs, therapy-related myeloid neoplasms; PARPi, poly(ADP-ribose) polymerase inhibitor.

- The authors state that one of the key findings is that t-MN incidence was higher than "typically" observed. What exactly is meant by this? Higher than the t-MN rate after PARPi or the overall t-MN rate? Please clarify. It would also be informative if the authors could include a table with the t-MN incidence rates after PARPi reported in other studies.

Response: Thank you for your valuable comments. We have replaced the term "typical" with "overall incidence of t-MNs." In a previous study analyzing 23,862 patients with ovarian cancer in the United States, the incidence of t-MNs was reported to be 0.277% (13). However, in various prospective and retrospective studies involving PARPi treatment for ovarian cancer, the incidence of t-MNs was shown to be higher, ranging from 1.53% to 3.81% (5, 6, 14, 15, 22). In response to your suggestion, we have added a table to present this information (Supplementary Table 1).

"The incidence of t-MNs after PARPi therapy in the current study was higher than that of overall t-MNs" (Abstract, Page 3, Line 18-19)

"and that following PARPi therapy was 1.53%–3.81% (5,6,14,15,22) (Supplementary Table 1)." (Discussion, Page 11, Line 20-21)

Supplementary Table 1. Characteristics of studies on t-MNs occurring after PARPi therapy

	Types of PARPi	Cancer type	All patient numbers	Number of patients diagnosed with t-MNs	Previous chemotherapy
Matulonis, et al (2023) [15]	Niraparib	Ovarian cancer	367	14 (3.81%)	Two
Marmouset V et al (2022) [14]	Olaparib, Niraparib, Rucaparib, Talazoparib	Ovarian cancer	373	13 (3.48%)	Two
DiSilvestro P et al (2023) [6]	Olaparib	Ovarian cancer	260	4 (1.53%)	One
Poveda A et al (2021) [22]	Olaparib	Ovarian cancer	195	6 (3.07%)	At least two
O'Malley DM et al (2022) [5]	Rucaparib	Ovarian cancer	375	14 (3.73%)	At least two

t-MNs Therapy-Related Myeloid Neoplasms; PARPi, (Poly ADP Ribose Polymerase inhibitor);

- The authors state that they did not identify PARPi as a predictor of t-MN occurrence. This is expected as all patients received PARPi treatment and no control group was included in the study. I suggest removing this statement.

Response: Accordingly, we have removed the sentence "However, we did not identify PARPi therapy as a significant predictor of t-MN occurrence."

- The reported t-MN incidence rate in this study is indeed very high, although this has to be interpreted in view of the small patient number (only 5 cases). Moreover, the incidence is strongly dependent on the patient cohort, particularly on the previous treatment history. This should be discussed in the discussion section.

Response: Thank you for the comment. In our study, although t-MNs occurred in 4 patients, the overall incidence of 7.7% was relatively high compared to previously reported studies, considering the total number of 52 treated patients. While factors such as cumulative doses of cytotoxic chemotherapy, BRCA status, and prior CHIP variants could be considered, the small sample size made it difficult to reach statistical significance. Additionally, because some patients had an overall survival exceeding 20 years after ovarian cancer diagnosis, we were unable to obtain data on the cumulative doses of cytotoxic chemotherapy for some patients, and therefore, this analysis could not be performed. This limitation has been discussed in the manuscript.

"In our study, the incidence of t-MNs after PARPi therapy was 7.7%, which is higher than the incidence of 1.53–3.81% reported in previous studies (5,6,14,15,22). Several factors could account for the higher incidence of t-MNs, such as prior treatment history, BRCA status, and prior CHIP variants. However, we were unable to identify any statistically significant factors. Additionally, the occurrence of t-MNs is notably influenced by prior treatment history. Therefore, including the cumulative doses of cytotoxic chemotherapy

and radiotherapy in the analysis would have been valuable. However, owing to missing data for some patients, this analysis could not be performed." (Discussion, Page 13-14, Line 20-6)

Reviewer B

Numerous papers has been published in this setting but this is the first including Korean patients.

Major :

Authors have to clarify if Case 1 is a t-MN or not as there is no bone marrow aspiration or biopsy, no cytogenetic or molecular characterization and it is not clear if the blast were pathological. It Myeloblast or erythroblast ? A control at distance of Parpi stop has been done ? The absence of deep cytopenia and the absence a clear myeloid characterization should lead the authors to not include this case as a t-MN.

Response: Thank you for your valuable comments. In the case of Case 1, myeloblasts were clearly suspected based on the peripheral blood smear, and it is clinically considered a therapy-related MN. Additionally, in Case 1, deep cytopenia with hemoglobin levels below 6 g/dL and platelet counts below 20,000/μL was observed over a period of 5 months, leading to the patient's death following the discontinuation of PARPi. Furthermore, a subsequent peripheral blood smear consistently showed blasts at 20% or more. However, considering the lack of evidence for cytogenetic or molecular characterization through examinations such as bone marrow biopsy or immunophenotyping, Case 1 has been excluded. Additionally, this has been included as part of the exclusion criteria.

"cases with insufficient cytogenetic abnormalities and molecular characterization for diagnosing t-MNs." (Methods, Page 7, Line 19-20)

Minor :

-More than discussing REF 15 with only 9 t-MNs, authors could discuss REF 14 with 69 t-MNs, to describe TP53 mutation rate in t-MN.

Response: We appreciate your valuable comments. Following your suggestion, we have changed the reference for the TP53 mutation rate and revised the sentence as follows.

"This result is consistent with a prior study that reported a TP53 mutation rate of 71.1% in 69 t-MN patients following PARPi therapy." (Discussion, Page 13, Line 10-12)

-Authors could discuss the interest of NGS sequencing for Ovarian patients under PARPi treatment as papers has described high incidence of CHIP in this setting and cite these 2 papers

Kwan TT, Oza AM, Tinker AV, Ray-Coquard I, Oaknin A, Aghajanian C, Lorusso D, Colombo N, Dean A, Weberpals J, Severson E, Vo LT, Goble S, Maloney L, Harding T, Kaufmann SH, Ledermann JA, Coleman RL, McNeish IA, Lin KK, Swisher EM. Preexisting TP53-Variant Clonal Hematopoiesis and Risk of Secondary Myeloid Neoplasms in Patients With High-grade Ovarian Cancer Treated With Rucaparib. JAMA Oncol. 2021 Dec 1;7(12):1772-1781. doi: 10.1001/jamaoncol.2021.4664. PMID: 34647981; PMCID: PMC8517887.

Martin JE, Khalife-Hachem S, Grinda T, Kfoury M, Garciaz S, Pasquier F, Vargaftig J, Uzunov M, Belhabri A, Bertoli S, Cotteret S, Vergé V, Renneville A, Rosselli F, Antony-Debre I, Rouleau E, Salviat F, Caron O, Delaloge S, Pautier P, Etienne G, Recher C, Vey N, De Botton S, Leary A, Marzac C, Micol JB. Therapy-related myeloid neoplasms following treatment with PARP inhibitors: new molecular insights. Ann Oncol. 2021 Aug;32(8):1046-1048. doi: 10.1016/j.annonc.2021.04.015. Epub 2021 Jun 6. PMID: 34107346.

Response: Thank you for your valuable comments. Accordingly, we have added the following sentence to the Discussion section, along with the recommended references, regarding the potential association between preexisting CHIP variants and the development of t-MNs after PARPi treatment.

"Furthermore, several studies have reported an association between preexisting clonal hematopoiesis of indeterminate potential (CHIP) variants and the development of t-MNs following PARPi treatment (26, 27, 28). These studies suggest that PARPi therapy imposes selective pressure, leading to the enhanced expansion of specific clones, particularly those with *TP53* mutations (27). Therefore, performing NGS to identify CHIP variants before starting PARPi treatment could provide a clearer understanding of the associated risks."
(Discussion, Page 13, Line 14-19)

Ref)

26. Kwan TT, Oza AM, Tinker AV, et al. Preexisting TP53-variant clonal hematopoiesis and risk of secondary myeloid neoplasms in patients with high-grade ovarian cancer treated with Rucaparib. JAMA Oncol 2021;7:1772-81.

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28. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature 2015;518:552-5.